

The effects of alendronate in the treatment of experimental osteonecrosis of the hip in adult rabbits

J. G. Hofstaetter M.D.^{†‡*}, J. Wang M.D., Ph.D.^{†§}, J. Yan M.D.[†] and M. J. Glimcher M.D.[†]

[†] Children's Hospital Boston, Harvard Medical School, 300 Longwood Avenue, Boston, MA 02115, USA

[‡] Department of Orthopaedics, Vienna General Hospital, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria

[§] Harrington Laboratory for Molecular Orthopedics, Department of Orthopedic Surgery, University of Kansas Medical Center, Kansas City, KS 66160, USA

Summary

Objective: Characterize the effects of alendronate (ALN) on the repair process of the osteonecrotic femoral head as well as the development of secondary osteoarthritis in the ipsilateral hip in an established experimental model of osteonecrosis.

Methods: Osteonecrosis of the femoral head was induced surgically in 60 adult, male New Zealand white rabbits. Animals were randomized in two placebo- (saline) and two treatment-groups (ALN 150 µg/kg/day S.C., 3× per wk) and were euthanized at 6 and 12 months post-operatively. Contralateral hip was used as control. Micro-Quantitative-CT (µQCT) analysis as well as histological assessment was performed in the femoral head and the acetabulum. Mankin Score was used to assess cartilage degeneration in the acetabulum.

Results: Repair in the osteonecrotic femoral head in the placebo group led to a significantly increased bone volume fraction (BVf) and volumetric bone mineral density (vBMD) in the trabecular region and to an increase in porosity in the cortical and subchondral region when compared to the normal femoral head on the contralateral side. ALN treatment significantly further increased BVf and vBMD in the trabecular region, and significantly reduced porosity and increased vBMD in the necrotic subchondral and cortical bone when compared to placebo. ALN led to a significant increase in vBMD in the subchondral region of the osteoarthritic acetabulum as well as to a significant reduction in articular cartilage degeneration.

Conclusion: Inhibition of bone resorption by ALN treatment during repair of the osteonecrotic femoral head significantly increased bone mass in the trabecular region of the femoral head, inhibited subchondral resorption and reduced cartilage degeneration in the acetabulum.

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Key words: Osteonecrosis, Micro-computed tomography, Femoral head, Osteoarthritis, Alendronate.

Introduction

Osteonecrosis of the femoral head is a common disease in a relatively young patient population, with an average age of 36 years^{1,2}. If left untreated, the disease progresses and can ultimately lead to the collapse of the femoral head and hip joint destruction^{3,4}. In the early stage of the disease joint-preserving surgical techniques are often considered, which also have a significant failure rate and morbidity^{5,6,7,8}. Total hip arthroplasty (THA) is often the procedure of choice in the treatment of osteonecrosis of the hip⁷. However, THA may be not an attractive option for this young patient population. It is desirable to avoid or at least delay THA, because most of these young patients outlive the current state-of-the art prostheses and some studies suggest less than satisfactory results of hip arthroplasty in this patient population^{6,9}.

The pathogenesis that leads to the collapse of the femoral head with subsequent hip joint destruction is not entirely clear. The mechanical behavior of a structure depends on the material and geometric properties of the structure¹⁰. Bone cell death *per se* does not change the trabecular architecture, and may not on its own explain the structural failure of the femoral head. Results from earlier studies^{11,12,13,14} suggested that the resorption of necrotic bone, especially compact bone during the repair process may play a role in the weakening of the structural properties of the femoral head. If bone resorption associated with osteonecrosis can be inhibited or delayed until sufficient new bone has formed, it would appear that structural failure could be delayed or avoided. Bisphosphonates, synthetic analogues of pyrophosphate, have emerged as valuable drugs in the treatment of osteoporosis and Padgett's disease¹⁵. ALN has been shown to prevent resorption of necrotic bone during revascularization without impairing new bone formation in a bone chamber study in rats¹⁶. Recent studies have shown very promising results with bisphosphonate treatment in experimental Perthes disease^{17,18} as well as in traumatic osteonecrosis of the femoral head in adolescents¹⁹. Although there are already data from clinical studies reporting promising preliminary results using bisphosphonates in the treatment of osteonecrosis of the femoral head in

*Address correspondence and reprint requests to: Dr Jochen G. Hofstaetter, M.D., Department of Orthopaedics, Vienna General Hospital, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. Tel: 43-1-40400-4060; Fax: 43-1-40400-4088; E-mail: jochen.hofstaetter@meduniwien.ac.at

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adults^{20,21,22}, there are surprisingly little experimental data on the effects of bisphosphonates in the treatment of adult osteonecrosis. Moreover, previously reported experiments only evaluated the femoral head and did not address secondary changes in the ipsilateral acetabulum^{17,18}.

In recent years, micro-computed tomography (μ CT) has been shown to be a sensitive tool in characterizing three-dimensional (3-D) bone microarchitecture and in various conditions and explaining mechanisms of bone failure²³. μ CT has been shown to be more sensitive to changes in bone microstructure than conventional histomorphometry²⁴ and has been used very successfully to characterize bony changes in osteoarthritis^{25,26,27} as well as experimental osteonecrosis¹⁴.

In this study we used Micro-Quantitative-CT (μ QCT) and histology to characterize the long term effects of ALN on the repair process of the osteonecrotic femoral head as well as the development of secondary osteoarthritis in the ipsilateral acetabulum, in an established experimental model of osteonecrosis in adult male rabbits¹⁴.

Material and methods

ANIMAL MODEL

Sixty unselected 10–12 month old, male New Zealand white rabbits were purchased from a USDA-licensed dealer (Millbrook ImmunoServ, Inc., Amherst, MA, USA). Rabbits were housed in single cages in a 12/12 h light/dark cycle and fed with standard Purina Chow in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The animal use protocol was approved by the Animal Care and Use Committee at the Children's Hospital Boston, MA. The animal model was described earlier in detail¹⁴. In brief, the animals were premedicated with 0.05 mg/kg glycopyrolate IM, 10 mg/kg ketamine IV and 0.5 mg/kg acepromazine IV. General anesthesia was maintained with the use of 2–3% of isoflurane with oxygen via an endotracheal tube. Under aseptic conditions, a posterior approach to the left hip was used. The hip joint capsule was completely removed and the periosteum and blood vessels covering the femoral neck were cauterized circumferentially to interrupt the blood supply to the femoral head. The ligamentum teres was then ligated with a nonresorbable suture (Ethibond Excel 5.0[®], Ethicon GmbH, D-22851 Nordstedt, Germany). A 3-mm drill hole was made through the posterior femoral neck into the marrow cavity, which was then cauterized to interrupt the intramedullary blood supply to the femoral head. Prophylactic antibiotics (25 mg/kg cefazolin IV) were given preoperatively and 2 h post-operatively. The rabbits were allowed full weight-bearing post-operatively. Post-operative pain was managed with IM injection of 0.03 mg/kg buprenorphine q 12 h buprenorphine twice daily for 3 days post-operatively. The left hip in all of the animals was used for the production of osteonecrosis, the right non-operated hip served as a control.

TREATMENT GROUPS

Sixty rabbits were randomized into four groups of 15 animals each. Two treatment-groups received ALN at a dose of 150 μ g/kg/day (s.c., 3 \times per wk) and two placebo groups received normal saline-injections (s.c., 3 \times per wk) starting 1 month post-surgery. The dosage of ALN was recommended by Gideon Rodan, Ph.D., Merck & Co., Inc. The dosage corresponds to the oral dosage in humans for osteoporosis treatment. Due to the low oral bioavailability of bisphosphonates in human, the dosage the rabbits received is estimated to be at least 50-fold higher. Rabbits of the placebo group and the treatment group were sacrificed at 6 and 12 months post-surgery each with an i.v. injection of Fatal Plus[™]. The time-points 6 and 12 months were chosen, based on our previous publication using the same animal model¹⁴, in which we found very little signs of repair at 4 weeks after surgery, but a marked repair response at 6 months.

TISSUE HARVEST AND IMAGING

Bilateral proximal femora and acetabuli from the placebo groups and the osteonecrotic femoral head with the ipsilateral acetabulum from the ALN groups were harvested and fixed in 10% neutral buffered formalin (NBF) for 7 days. Samples were then placed in a special polycarbonate specimen tube filled with distilled water and scanned by a cone beam μ QCT system (GE Healthcare BioSciences). Data sets with isotropic 18 μ m voxel spacing

were acquired at 0.5 $^\circ$ steps over a total rotation of 360 $^\circ$ at 80 kVp (plus 0.5 mm of Al and 15 mm of acrylic added filtration). The specimen tube was surrounded by an acrylic field flattener (21 mm thick) in order to minimize beam hardening. A polycarbonate calibration phantom containing 2.3 mm diameter cylinders of air, water and SB3, a hydroxyapatite (HA) mimicking material, (Gammex RMI, Middleton, WI) was scanned in order to scale values of CT attenuation to bone mineral density (BMD). A linear model consisting of a two-point calibration was used to map individual voxel intensity values to BMD. The mean attenuation of water represented 0 mg/cc of HA while the mean attenuation within the SB3 calibrator represented a density of 1030 mg/cc of HA. Using two-point calibration, attenuation values were both interpolated and extrapolated to determine volumetric bone mineral density (vBMD, mg mineral/cc).

Images were reconstructed into 3-D volumes using true Feldkamp reconstruction with 16-bit gray levels. Unbiased bone volume fraction (BVf) in the trabecular region was calculated from the 3-D images using MicroView[®] software (GE Health Care). Thresholds for each specimen were chosen to separate bone from marrow by determining the HU value that maximized the between class variance of water and bone values in the μ CT scans²⁸.

Measurements were taken in standardized positions in four different regions (anterior, posterior, medial, lateral) in the cortical bone of the femoral neck (cross-section) and in five regions in the subchondral bone of the femoral head (sagittal-section) and values were averaged. Anatomical landmarks and length measures were used for orientation. At each position apparent porosity (percentage of void volume in the cortex; porosity = 1 – BVf) and vBMD (mg mineral/cc) were determined. In the trabecular region of the femoral head bone a 9 mm³ volume of interest (VOI) was positioned in the center of the femoral head and microstructural parameters mentioned above as well as vBMD were determined. Thickness, vBMD and apparent porosity were determined in the subchondral bone of the central portion of the weight-bearing region of the acetabulum. Femoral head sphericity was determined using μ CT images. A femoral head was considered collapsed when a clear fracture line and/or femoral head deformation was present.

HISTOLOGY

After scanning, the specimens were decalcified with 25% formic acid containing 10% NBF, processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin and Safranin-O. The anterior and superior regions of the acetabulum were analyzed for the degree of histological change using the Mankin Score²⁹ modified as previously published³⁰. All sections were graded by two observers (JGH, JY) blinded to the group, and median scores were determined for statistical analysis. The capability of the system has been demonstrated, and it has been found useful for systematic assessment of articular cartilage, as the categories included in the scoring system encompass highly relevant histological and histochemical variables^{29,30}.

STATISTICAL ANALYSIS

Data and graphs are presented as mean \pm SD. Paired Student *t* test (two-tailed) was used to evaluate significance between placebo-operated and placebo-non-operated samples. Unpaired Student *t* test (two-tailed) was used to evaluate significance between the placebo-operated and ALN-operated samples. Fisher's exact test was used to evaluate statistical significance between placebo-operated and ALN-operated samples with regard to the collapse of the femoral head. All calculations were performed using Graph Pad Prism 4 for Windows (GraphPad Software, San Diego, CA, USA) and statistical significance was set at the 95% confidence limit.

Results

FEMORAL HEAD

Six months post-surgery, no collapse of the osteonecrotic femoral head was seen in the placebo group (0/15) and in the ALN group (0/15). At 12 months post-surgery 2 out of 15 (13.3%) osteonecrotic femoral heads were collapsed in the placebo group, whereas no collapse was seen in the ALN group (0/15). However, this difference was not statistically significant. Representative μ CT images of the femoral head and neck regions at 12 months post-surgery as well as of a collapsed femoral head are presented in Fig. 1. No difference in femoral sphericity was seen in the non-collapsed femoral heads (data not shown).

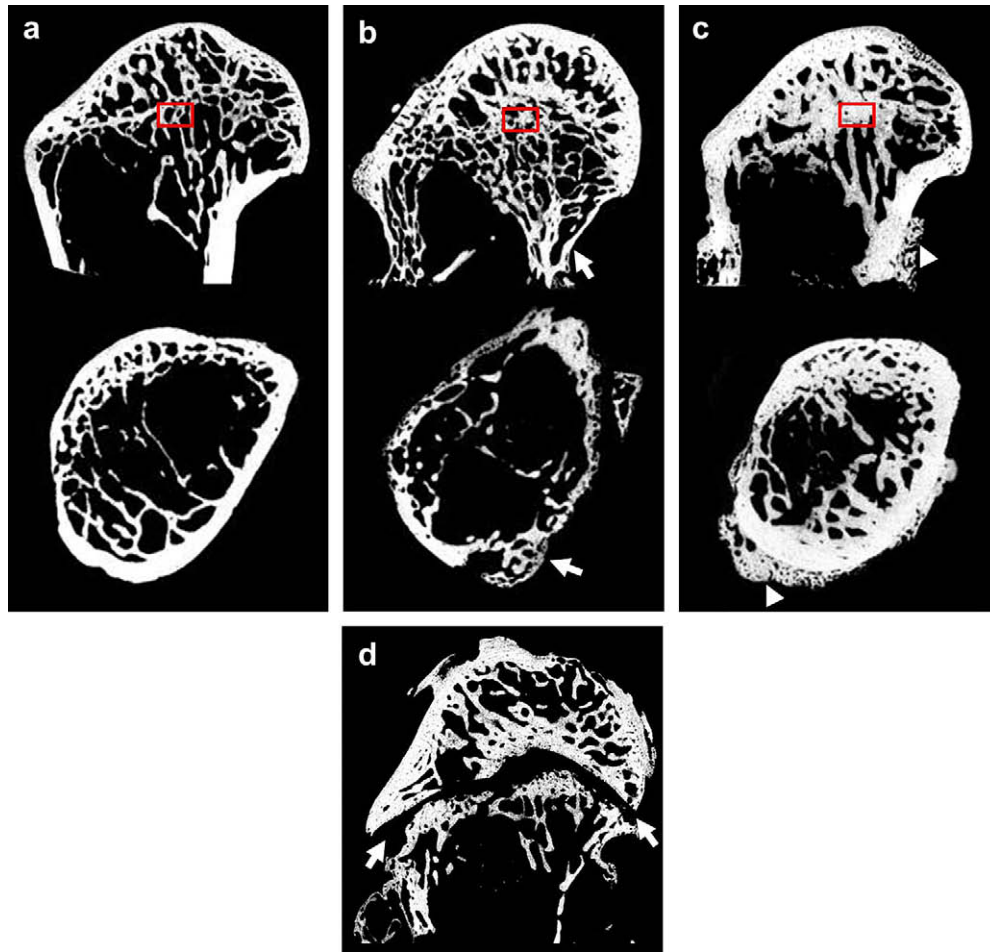


Fig. 1. Representative μ CT images of the femoral head (top) and the neck (bottom) at 12 months post-surgery. (a) Placebo group – non-operated femoral head, (b) placebo group – operated femoral head (arrow = area of bone resorption), (c) ALN group – operated femoral head (arrowhead = appositional bone formation + absence of bone formation), and (d) placebo group – collapsed operated, femoral head (arrow = fracture line). Red square in a–c indicates region seen on histological sections in Fig. 3.

Subchondral region of the femoral head [Fig. 2(a and b)]

At 6-month post-surgery, no significant changes in porosity and vBMD were seen between the groups. At 12 months, vBMD of the osteonecrotic femoral head in the placebo group was significantly decreased by 11.28% ($P < 0.05$) and porosity was increased 3.86-fold ($P < 0.0001$) when compared to their contralateral non-operated side. ALN treatment led to a 2.33-fold reduction ($P < 0.005$) in porosity and a 13.37% ($P < 0.05$) increase in vBMD when compared to placebo in the operated femoral heads.

No significant difference in vBMD and porosity was found between the osteonecrotic femoral head of the placebo group and the osteonecrotic femoral head of the ALN group at 12 months.

Trabecular region of the femoral head [Fig. 2(c and d)]

At 6 months and 12 months post-surgery, the repair process in the osteonecrotic femoral head in the placebo group led to a significantly increased BVF (15.05%, $P < 0.005$ and 13.71%, $P < 0.05$, respectively) and a significantly increased vBMD (9.19%, $P < 0.05$ and 17.64%, $P < 0.005$, respectively), when compared to its contralateral

non-operated side. Six months and 12 months ALN treatment significantly further increased BVF (15.5%, $P < 0.05$ and 31.16%, $P < 0.05$, respectively) and vBMD (20.99%, $P < 0.005$ and 30.51%, $P < 0.005$, respectively) in the osteonecrotic femoral head when compared to placebo. ALN treatment resulted in 32.91% ($P < 0.0001$) and 49.14% ($P < 0.0001$) higher BVF and 32.11% ($P < 0.0001$) and 53.53% ($P < 0.0001$) higher vBMD in the osteonecrotic femoral head when compared to the non-operated femoral head of the placebo group at 6 and 12 months, respectively.

Histological observations revealed different stages of repair process in the trabecular region of the osteonecrotic femoral head. During the repair process of osteonecrosis new appositional bone formation occurs on the surface of dead trabecular bone, following invasion of fibrovascular repair tissue, which is later remodeled (Fig. 3). ALN treatment does not appear to inhibit bone formation in the repair process (Fig. 3).

Cortical region of the femoral neck [Fig. 2(e and f)]

At 6 and 12 months post-surgery, porosity was significantly increased (2.59-fold ($P < 0.0001$) and 3.41-fold ($P < 0.0001$), respectively) and vBMD was significantly decreased (–30.35%, $P < 0.05$ and –39.7%, $P < 0.005$,

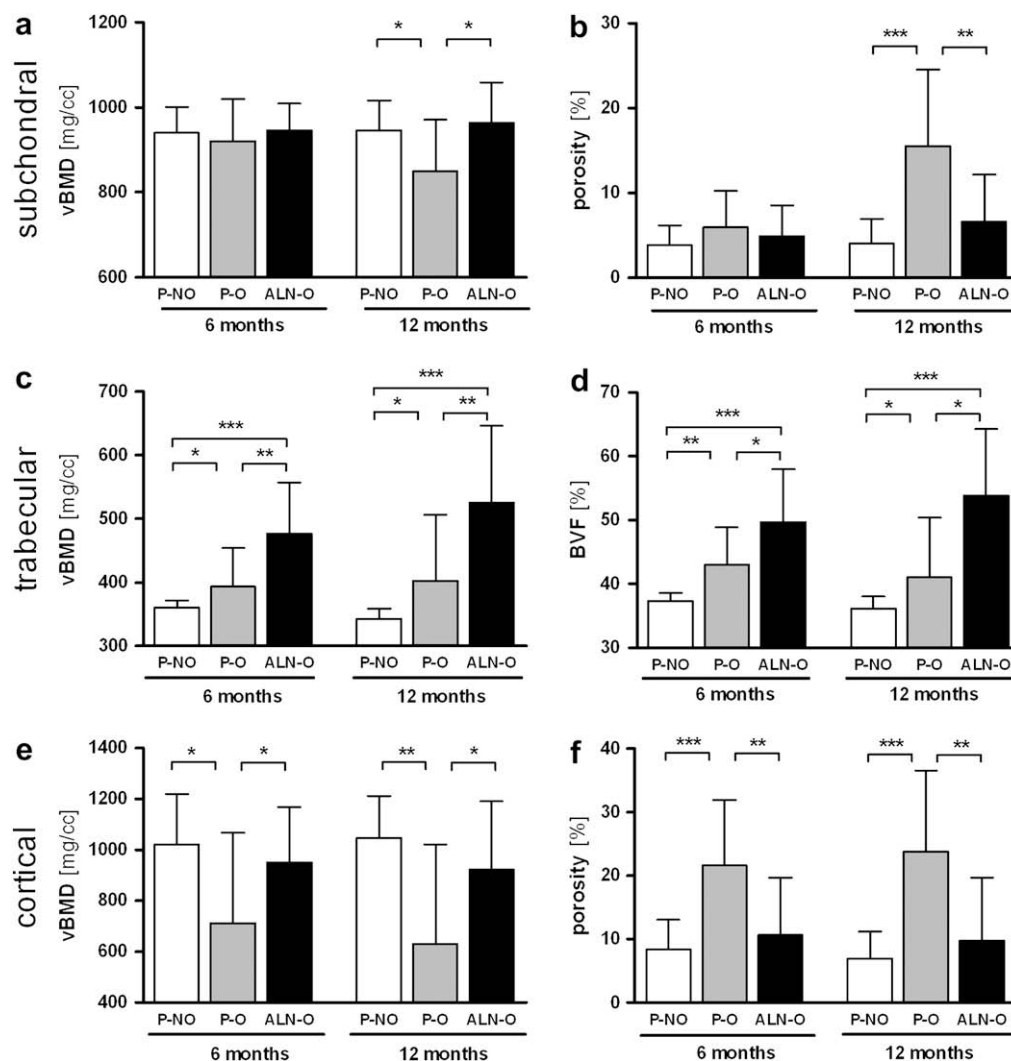


Fig. 2. μ QCT analysis of the femoral head of rabbits. (P-NO: placebo group – non-operated femoral head; P-O: placebo group – operated femoral head; and ALN-O: ALN group – operated femoral head) * indicates $P < 0.05$; ** indicates $P < 0.005$; and *** indicates $P < 0.0001$.

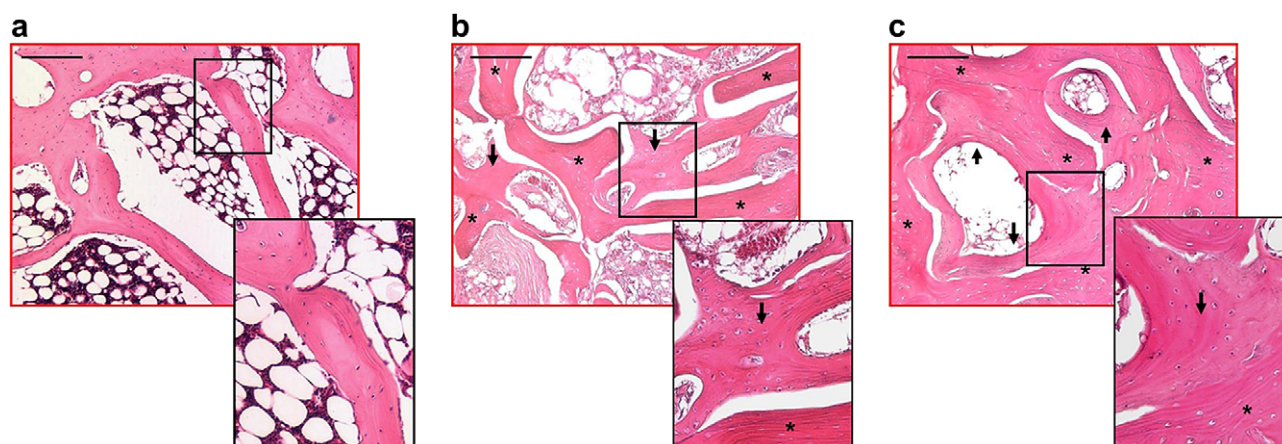


Fig. 3. Representative histological sections (areas are marked with red square in Fig. 1) showing that ALN treatment inhibits resorption of necrotic bone without inhibiting new bone formation. (a) Placebo group – non-operated femoral head, (b) placebo group – operated femoral head, and (c) ALN group – operated femoral head, (arrow marks appositional bone formation, * marks necrotic bone). Length of scale bar is 120 μ m.

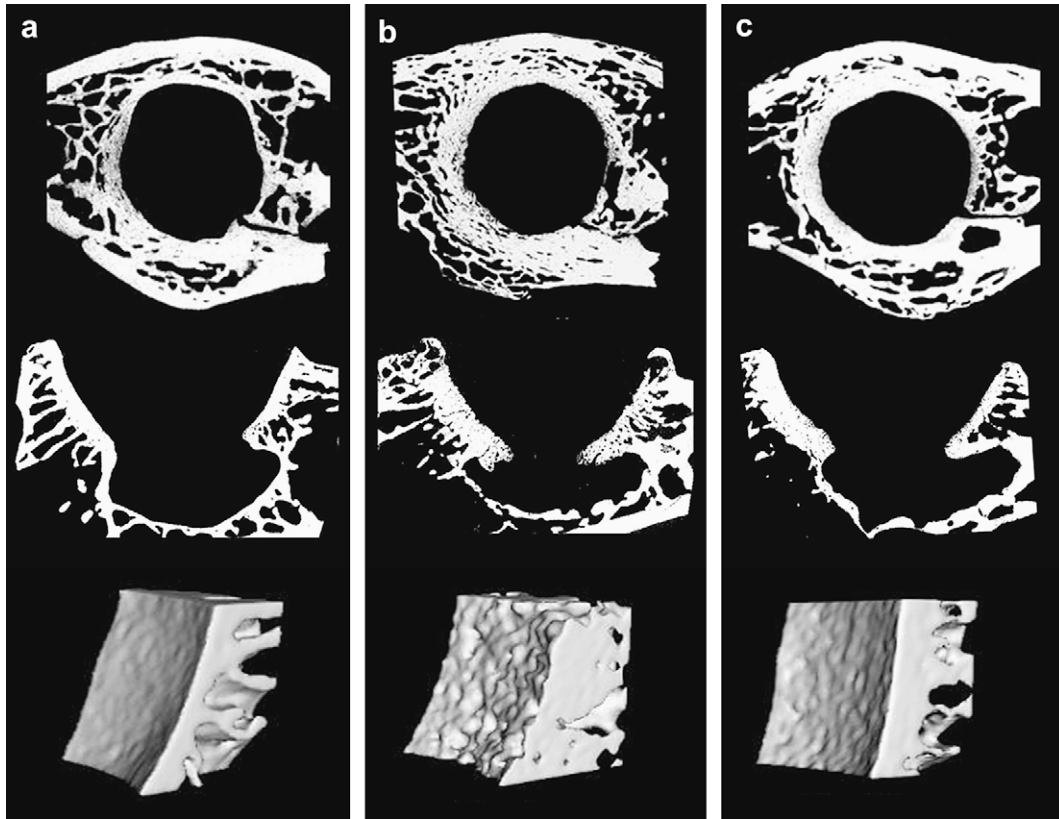


Fig. 4. Representative μ CT images of the acetabulum (direct view – top row; lateral view – middle row; and 3-D reconstruction of chondral/subchondral region) at 12 months post-surgery. (a) Placebo group – non-operated femoral head, (b) placebo group – operated femoral head, and (c) ALN group – operated femoral head.

respectively) in the cortex of the osteonecrotic femoral neck of the placebo group when compared to their contralateral control side. At 6 months and 12 months ALN treatment led to a significant reduction in porosity (-50.78% , $P < 0.005$ and -58.8% , $P < 0.005$, respectively) and a significant increase in vBMD ($+33.6\%$, $P < 0.05$ and $+46.5\%$, $P < 0.05$, respectively) in the osteonecrotic femoral head when compared to the placebo group. No statistically significant difference was found between the ALN treated osteonecrotic femoral neck and non-operated femoral heads of the placebo group.

ACETABULUM

In the placebo group, the acetabular subchondral bone of the operated hip was significantly thicker (9.3% , $P < 0.05$ and 17.33% , $P < 0.001$, respectively) with a significantly higher porosity (1.82-fold, $P < 0.05$ and 3.67-fold, $P < 0.0001$) and a decreased vBMD (-8.03% , $P < 0.005$ and -13.26% , $P < 0.005$) when compared to the contralateral non-operated side at 6 and 12 months post-surgery. Representative μ CT images of the acetabulum at the 12-month time-point are presented in Fig. 4. In the acetabulum of the ALN-treated operated hip, the subchondral bone had a significantly higher vBMD ($+4.45\%$, $P < 0.05$) than the acetabulum of the placebo treated operated hip at 6 months, while no differences were found with regard to thickness and porosity. However, the 12 months ALN treatment led to a significant 55.56% ($P < 0.005$) reduction in porosity and a significant 11.53% ($P < 0.05$) increase in vBMD in the acetabulum of the operated hip when compared to the

placebo group. Subchondral bone of the ALN-treated operated hip was significantly thicker ($+5.3\%$, $P < 0.05$) at 6 months and 10.67% ($P < 0.05$) thicker at 12 months than that of the non-operated hip of the placebo group and had a significantly higher porosity ($+63.2\%$, $P < 0.05$) at 12 months. All μ CT data are presented in Fig. 5.

HISTOLOGICAL GRADING ARTICULAR CARTILAGE ACETABULUM

At 6 months, histological grading using the Mankin Score²⁹ of the articular cartilage of the acetabulum of the operated hip showed that the placebo group (7.5 ± 2.6 , $P < 0.0001$) as well as the ALN group (5.8 ± 2.1 , $P < 0.0001$) developed secondary osteoarthritis when compared to the non-operated hip of the placebo group (0.3 ± 0.2). Although the operated hip of the placebo group showed more degeneration than the ALN group, the difference was not significant ($P < 0.058$) at 6 months. At 12 months, however, there was a significantly higher degree of degeneration in the operated hip of the placebo group than in the ALN group (14.5 ± 4.3 vs 9.7 ± 3.6 , $P < 0.005$) (Fig. 4d). Representative histological sections at 12 months post-surgery are presented in Fig. 6(a–c).

Discussion

In this study we investigated the effects of high-dose ALN treatment on the repair of osteonecrotic femoral heads as well as the development of secondary osteoarthritis of the

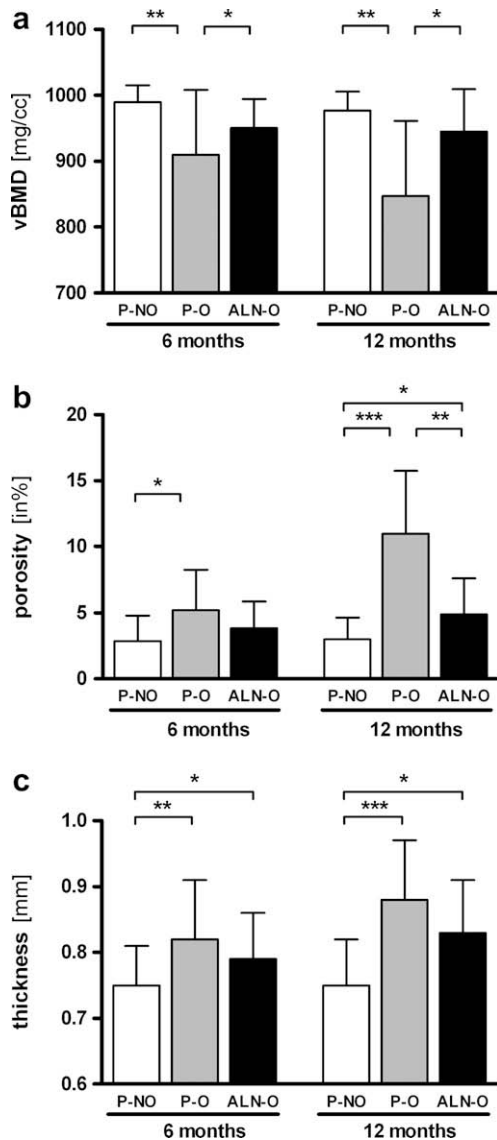


Fig. 5. μ QCT analysis of subchondral bone of the acetabulum (anterior region) in rabbits. (P-NO: placebo group – non-operated acetabulum; P-O: placebo group – operated acetabulum; and ALN-O: ALN group – operated acetabulum) * indicates $P < 0.05$; ** indicates $P < 0.05$; and *** indicates $P < 0.0001$.

ipsilateral acetabulum in an experimental model of osteonecrosis in adult rabbits. In the placebo group we observed a significant increase in BVF and vBMD at 6 months post-operatively during the normal repair process in the trabecular region of the osteonecrotic femoral head when compared to the contralateral control side. This increase in bone mass can be explained by appositional new bone formation on the surface of the dead trabeculae during the repair process, consistent with histological findings. Morphological evaluations of the rabbit model of osteonecrosis used in the present study has been described in the previous publication¹⁴. In brief, at 4 weeks after the surgical induction of osteonecrosis of the femoral head, the infarcted femoral heads appeared yellowish and pale. Histological observations revealed that all of the operated femoral heads displayed extensive cell death in the marrow space and empty lacunae in most areas of the trabecular bone,

although the extent of osteonecrosis varied slightly among the animals. Repair responses were not evident at this time. Marked repair responses to the necrotic bone, such as revascularization and new bone formation on the surfaces of necrotic trabecular bone, were observed at 6 months after surgery. Kenzora *et al.* also reported appositional bone formation on top of dead trabecular bone in an experimental model of osteonecrosis in rabbits³¹. ALN treatment led to a significant further increase in BVF and vBMD in the osteonecrotic trabecular region when compared to placebo. Despite the fact that ALN treatment has been shown to decrease bone activation frequency³², our data indicate that ALN treatment did not impair new bone formation, but inhibited resorption of necrotic bone. Astrand *et al.* also showed in a bone chamber study in rats that ALN inhibited the resorption of necrotic bone, but did not impair bone healing¹⁶. Bisphosphonates have shown to have an initial positive effect on new bone formation in cortical healing, but did prolong the remodeling process³³. In contrast, resorption predominated the repair of dead compact cortical and subchondral in the placebo group, which led to a decrease in vBMD and an increase in apparent porosity. In our previous study using this animal model¹⁴, we found no significant decrease in vBMD and no increase in apparent porosity in the cortical bone of the femoral neck due to resorption at 4 weeks post-operative. However, we also found a significant reduction in vBMD and a significant increase in apparent porosity in the cortical femoral neck at 6 months post-operatively. These findings are in accordance with an earlier publication from Kenzora *et al.*³¹. Resorption of dead compact bone during the repair process of osteonecrosis may weaken the structural properties of the femoral head and may be in part responsible for the collapse seen in the late stages of osteonecrosis^{11,14,31,34}. ALN treatment significantly reduced the increase in porosity and reduced the decrease in vBMD in the compact bone. In this study, 12 months post-operative 13.3% (2/15) of the osteonecrotic femoral heads from the placebo group were collapsed, whereas no collapse (0/15) was seen in the ALN group, however, this result was not significant. Femoral head and neck geometry, the biomechanics of the hip joint as well as body weight are very different in rabbits when compared to humans, which may be critical with regard to the risk of femoral head collapse^{3,31}.

Recent studies by Kim *et al.*¹⁷ as well as Little *et al.*¹⁸ have shown very promising results with bisphosphonate treatment in experimental Perthes disease. They have shown that early treatment with ibandronate using a surgical piglet model¹⁷ and Zoledronic acid using a surgical rat model¹⁸ prevents femoral head deformity following experimental osteonecrosis of the juvenile femoral head. They found that repair process in the necrotic femoral heads, such as revascularization of the necrotic marrow space and proliferation of mesenchymal and fibroblastic cells, started as early as 2 weeks post-operation. Osteoclastic bone resorption, and femoral head flattening occurred as early as 4–8 weeks post-surgery. They started treatment at the time of surgery and animals were euthanized at 4 and 8 weeks¹⁷ and 6 weeks¹⁸ post-surgery. In contrast, repair response was not evident at 4 weeks after surgery in our rabbit model of osteonecrosis¹⁴, suggesting that repair process occurs much slower in the rabbit model than the piglet model. Therefore, we decided to start the bisphosphonate treatment at 4 weeks post-surgery, and not at the time of surgery.

Surgical osteonecrosis in a growing animal apparently produces a more rapid femoral head deformity than in adult

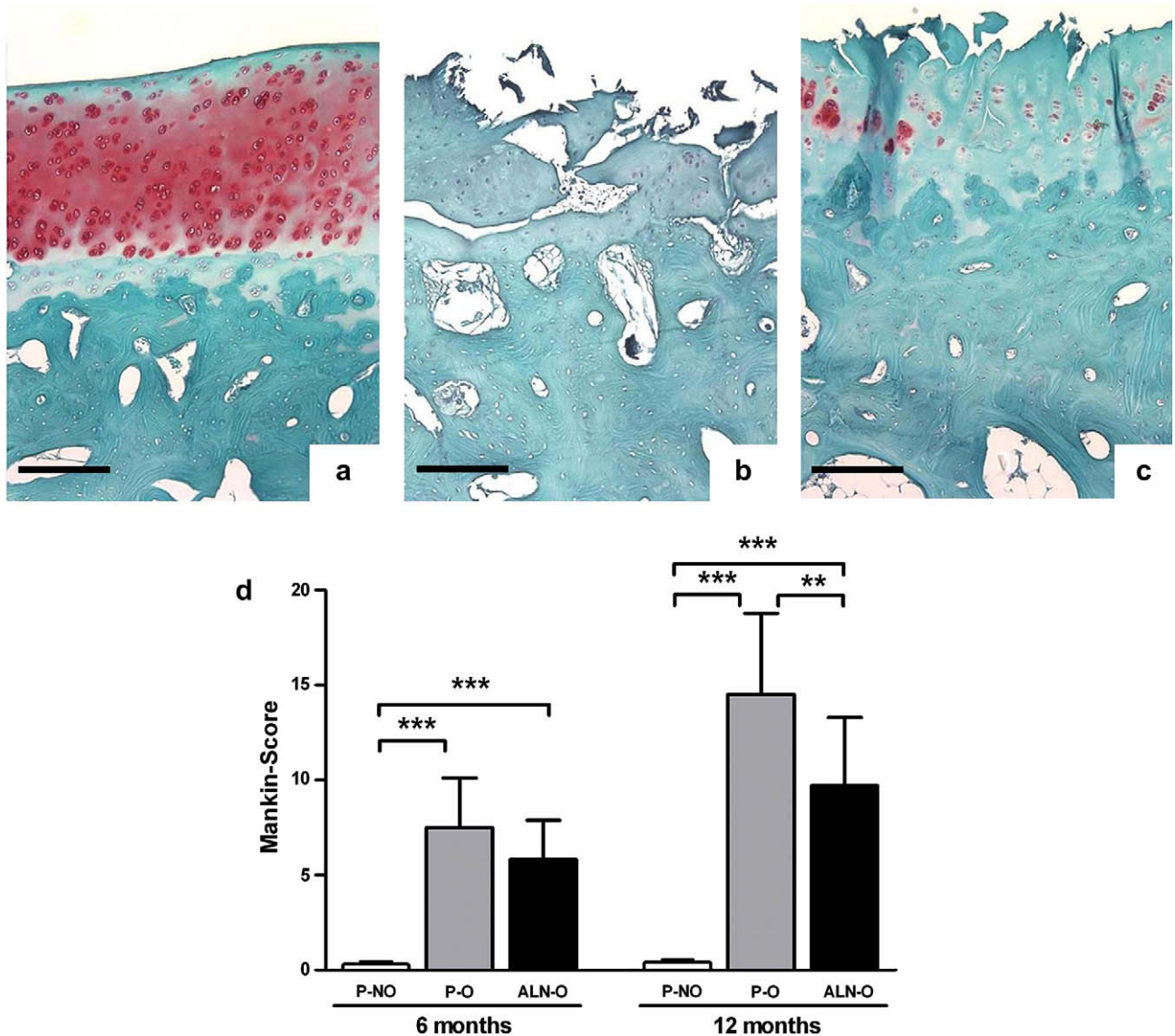


Fig. 6. Representative histological sections of the articular cartilage and subchondral region of the anterior acetabulum (Safranin-O staining) at 12 months post-surgery. (a) P-NO: placebo group – non-operated acetabulum, (b) P-O: placebo group – operated acetabulum, (c) ALN-O: ALN group – operated acetabulum, and (d) histological grading of articular cartilage degeneration using the Mankin Score. Length of scale bar is 120 μm. ** indicates $P < 0.05$ and *** indicates $P < 0.0001$.

animals. Interestingly, we also found an increase in BVF in the osteonecrotic femoral head during the repair process in this study as well as in our previous report¹⁴ at 6 months post-surgery, which is in contrast to the decrease in bone mass seen in the experimental Perthes model in piglets^{17,35}. These findings indicate that there is a difference in the repair process of necrotic bone between the ages of growth and adulthood.

Moreover, previous studies on experimental osteonecrosis only evaluated the femoral head and did not pay attention to the acetabulum. The degree of osteoarthritis in the acetabulum is a major determinant of whether a joint-preserving technique or total hip replacement is indicated. We found a significant increase in thickness, porosity and a decrease in vBMD in the subchondral bone of the osteoarthritic ipsilateral acetabulum. These changes in the osteoarthritic subchondral bone are in line with previously

published changes in osteoarthritic subchondral bone^{25,36}. ALN treatment led to a significant decrease in porosity with a significant increase in vBMD. Moreover, articular cartilage degeneration of the ipsilateral acetabulum of our operated animals was significantly less in the ALN treated group, when compared to the placebo group. However, we are not able to conclude whether the effects seen are secondary to the effects on the osteonecrotic femoral head, or due to direct effects on the acetabulum, or both. Resorption of subchondral bone may play a critical role in the development of osteoarthritis³⁷. Recent studies have shown that bisphosphonate-mediated inhibition of bone resorption with the subsequent decrease in bone turnover and the increase in bone mineralization does have a beneficial effect in the treatment of osteoarthritis^{38,39}. The finding of the development of osteoarthritis in the ipsilateral acetabulum prior to severe changes in femoral head sphericity

and/or collapse implies that other mechanisms than just mechanical may be involved in the disease process of osteoarthritis following osteonecrosis of the femoral head. Earlier studies reported differences in the synovial fluid composition between primary osteoarthritis and secondary osteoarthritis following osteonecrosis, which could differentially affect cartilage metabolism in the acetabulum^{40,41}.

Our surgical model of adult osteonecrosis represents traumatic osteonecrosis. Most experimental models of osteonecrosis are burdened by the fact that interventions are conducted in healthy animals. The etiology of osteonecrosis is very diverse, such as alcohol use, high-dose corticoid administration, coagulation abnormalities as well as genetic polymorphism which may predispose certain patient cohorts to the development of osteonecrosis³. These underlying diseases may affect bone repair and may also affect the efficacy of pharmacological agents in the treatment of osteonecrosis. High-dose bisphosphonate treatment was recently linked to the development of maxillary osteonecrosis after dental procedures in tumor patients^{42,43}. It is very likely that in patients with severe diseases such as cancer, the combination of chemotherapy with high-dose bisphosphonate treatment may be responsible for that finding.

Further experimental studies investigating the efficacy of bisphosphonates in various animal models of osteonecrosis are needed in the future. Moreover, it needs to be investigated if there are stage-specific differences in the efficacy of bisphosphonate treatment. Our results are very promising, that bisphosphonates may become an important part in the treatment of osteonecrosis. However, we ought to be very careful in selecting patients for bisphosphonate treatment in osteonecrosis and we have to specifically pay attention for side effects in the mandibular and maxillary regions. Moreover it would be ideal not only to decrease resorption but also to increase new bone formation during the repair of the osteonecrotic femoral head¹¹. Recent studies using cell-based therapies⁴⁴, gene-therapy⁴⁵, and also the addition of growth factors^{46–48} have yielded very promising results for the future treatment of osteonecrosis. Future studies are needed to explore the efficacy of combining several treatment modalities such as antiresorptive drugs, an osteogenic stimulus, and joint-preserving surgery for the treatment of early stage osteonecrosis of the femoral head.

Conflict of interest

All authors have no conflict of interest.

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